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Stable Parent Anions of Dopamine and Adrenaline: A New Form of Neurotransmitters

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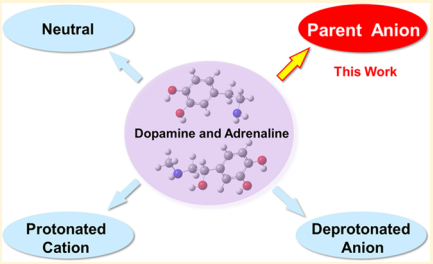
Supporting Information

ABSTRACT: Previously, dopamine and adrenaline were only known to exist in three closed-shell forms: neutral molecules (including zwitterions), protonated cations, and deprotonated anions. In the present work, stable open-shell parent anions of dopamine and adrenaline were generated in the gas phase and characterized by a combination of anion photoelectron spectroscopy and calculations. These anions were formed as a result of an enol−keto-type tautomerization initiated by the attachment of excess electrons. Calculations showed that hydrogen atoms on the hydroxyl groups of dopamine and adrenaline migrated to adjacent carbon atoms under the influence of the additional electron, breaking the aromaticity of the benzene ring and resulting in the formation of the rare anionic tautomers. We speculate that the secondary electrons generated in scenarios such as radiotherapy could produce the anions reported in this work, providing a potential new depletion channel of these molecules in vivo.



INTRODUCTION

Dopamine (D) and adrenaline (A) act as neurotransmitters and hormones in the nervous system. Due to their physiological effects, they are also widely used as medications.[1−3] These neurotransmitters act as ligands in interactions with their proprietary receptors. Knowing the structures of both the ligands and their receptors is crucial to understanding in vivo, lock-and-key recognition processes, with this knowledge sometimes leading to design of new drugs. While studies of the crystal structures of G-protein-coupled receptors for both dopamine and adrenaline have taught us much about them,[4−8] there is still more to learn about the forms in which dopamine and adrenaline can exist. Neutral dopamine and adrenaline are well known to occur as canonical or as zwitterionic molecules.[9] They can also exist as protonated cations due to the presence of lone pairs on the nitrogen atoms[10] and as deprotonated anions as a result of the acidic protons on their −OH groups.[11−13] As a matter of fact, these three forms (neutral, protonated cation, and deprotonated anion) are applicable to almost all of the biomolecules due to their stable closed-shell nature. The charge states of neurotransmitters can greatly influence their interaction with their receptors and therefore play important physiological roles. Furthermore, owing to their flexible molecular structures, both dopamine and adrenaline also possess a large number of conformations.[14−22]

Since gas-phase (in vacuo) studies deal with isolated molecular systems at relatively low temperatures and in simple environments free of outside influences, they have unique advantages in exploring and characterizing the various forms of these species. For dopamine, seven neutral conformers were discovered in the gas phase using Fourier transform microwave spectroscopy.[22] Moreover, theoretical calculations have been used to study the conformations of its protonated form in both the gas phase and solution.[23] Additional studies have focused on photoionization using femtosecond lasers.[24] Its deprotonated form was investigated via electrospray ionization coupled with tandem mass spectrometry.[12] For adrenaline, infrared photodissociation spectroscopy was utilized to study its protonated form.[10] Mass-selected ultraviolet/infrared hole burning spectroscopy[15] and theoretical calculations[25] were performed to study its neutral conformations. Deprotonated adrenaline was studied using nuclear magnetic resonance spectroscopy.[13] However, all of these studies were limited to closed-shell species, including neutral (zwitterionic) molecules and protonated or deprotonated ions. The parent (intact) anions of dopamine and adrenaline have not been observed.

In the present study, we report the formation and observation of the stable open-shell parent anions of both dopamine and adrenaline in the gas phase. These were characterized by a combination of time-of-flight mass spectrometry, anion photoelectron spectroscopy, and density functional theory (DFT) calculations. In these anions, we found that hydrogen atoms from their −OH functional groups had migrated to adjacent carbon atoms of the benzene ring, breaking its aromaticity and resulting in the formation of rare anionic tautomers with enhanced electrophilicity. This study thus introduces the previously unknown parent anions of

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dopamine and adrenaline. Under circumstances in which significant numbers of low energy electrons are present in vivo, these species could potentially exist there and have biological implications.

RESULTS

Experimental and theoretical methods are presented in the Supporting Information. The photoelectron spectra of the deprotonated dopamine anion (DD^-), parent dopamine anion (D^-), deprotonated adrenaline anion (DA^-), and parent adrenaline anion (A^-) recorded with 266 nm wavelength (4.66 eV) photons are presented in Figure 1. DD^- possesses an

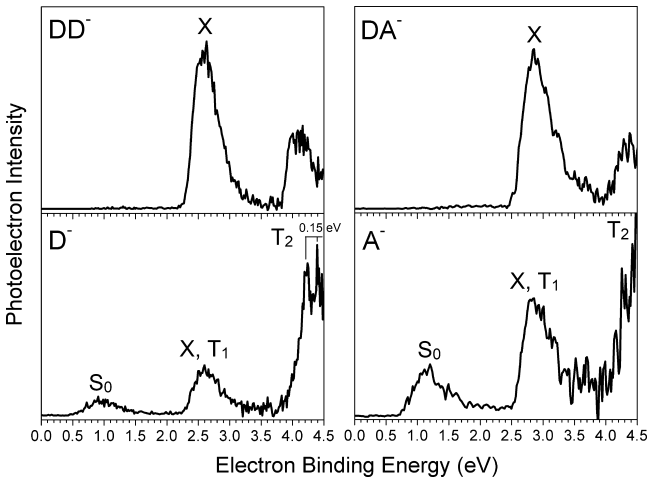

Figure 1. Photoelectron spectra of the deprotonated dopamine anion (DD^-), parent dopamine anion (D^-), deprotonated adrenaline anion (DA^-), and parent adrenaline anion (A^-). All spectra were taken with 4.66 eV photons.

electron binding energy (EBE) band with an onset of 2.3 eV and an intensity maximum at 2.54 eV where the latter (2.54 eV) is its experimental vertical detachment energy (VDE). The next higher EBE peak starts from ~3.85 eV and peaks at 4.10 eV, corresponding to a transition from the anion to the first excited state of its neutral counterpart. The spectrum of D^- shows several distinct features. The first EBE band starts from ~0.5 eV and peaks at 0.88 eV, which is its VDE value; it is marked as S_0, denoting the formation of a singlet (neutral) state post-photodetachment. The next higher EBE band (X, T_1), is due to a combination of mass coincidence with ^{13}C-containing DD^- and the transition to a higher triplet state (T_1) of the neutral. The fact that its intensity changed from day to day under different source conditions (Figure S1) further confirmed that this band has contamination from DD^-. The third EBE feature in the photoelectron spectrum of D^-, T_2, is a result of the transition to the neutral's next triplet state. T_2 starts from ~3.8 eV and spans the remainder of the spectrum. It comprises two peaks, that is, at EBE = 4.24 and 4.39 eV, and the spacing (0.15 eV) corresponds to the calculated ring shear vibration mode of the neutral species (1263 cm^{-1}) post-photodetachment. The lowest EBE band in the photoelectron spectrum of DA^- has an onset of 2.5 eV and an intensity maximum of 2.79 eV (VDE), while the next higher EBE peak starts from ~4.0 eV and spans the remainder of the spectrum. For A^-, the lowest EBE band (S_0) starts from ~0.7 eV and peaks at 1.10 eV (VDE). The second EBE band (X, T_1) is again due to mass coincidence from the ^{13}C-containing DA^- (Figure S1) and the transition to a triplet state of the neutral species. The third highest EBE band (T_2) starts from ~4.0 eV and peaks at 4.25 eV although there is some evidence of vibrational progression. All of the abovementioned experimental values are tabulated in Table 1 for comparison with the calculated values. The spectra of DA^- and A^- are similar to those of DD^- and D^-, which is expected since these two molecules have similar structures. Intuitively, the molecular orbitals involved in photodetachment are on the benzene moieties (vide infra), which are the same for both molecules, resulting in very similar spectra.

The calculated structures of neutral D and A, parent anionic isomers of D^- and A^-, are presented in Figure 2. The 3D

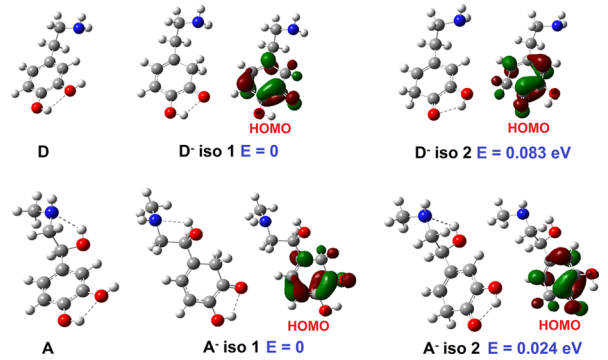

Figure 2. Calculated structures of neutral dopamine (D), parent dopamine anions (D^-) iso 1 and iso 2, neutral adrenaline (A), and parent adrenaline anions (A^-) iso 1 and iso 2. The highest occupied molecular orbitals (HOMO) of the anions are also presented.

coordinates of all the calculated species are listed in Table S1. For the neutral structures, the key to forming the most stable conformers is the intramolecular hydrogen bonding −OH → OH between the two hydroxyl groups on the catechol moieties in both cases and the intramolecular hydrogen bonding −OH → N in adrenaline (see the dotted H-bond interactions in Figure 2). Our calculations nicely reproduced the most stable conformers for neutral dopamine[22] and adrenaline[25] in previous studies. It is worth mentioning that non-tautomerized D^- and A^-, that is, those anions having very similar structures to their neutral counterparts, are 0.310 and 0.252 eV higher in

Table 1. Experimental and Calculated Vertical Detachment Energies and Higher Transitions of Parent Dopamine and Adrenaline Anions[a]

anion	expt VDE	cal VDE (S_0)		expt higher transitions	cal higher transitions (T_1, T_2)	
		iso 1	iso 2		iso 1	iso 2
D^-	0.88	0.754	0.747	2.54, 4.24	2.363, 3.715	2.396, 3.742
A^-	1.10	1.045	0.969	2.79, 4.25	2.653, 4.035	2.580, 3.960

[a]All numbers are in eV.

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energy than neutral D and A, respectively, suggesting that intact D and A cannot form anions and that tautomerization must be involved if a stable anion is to be formed.

The enigma of strong mass spectral signals of parent D^- and A^- observed in this study has been resolved by the combination of experiments and calculations. D and A each have two −OH groups on their catechol moieties from which hydrogen atoms have migrated, under the influence of the attached electrons, to an adjacent carbon atom on the benzene ring. This leads to two possible anionic isomers. In Figure 2, we labeled these anionic isomers iso 1 and iso 2. Our calculations showed that all of the anionic isomers have similar conformations to their neutral counterparts. In these anionic isomers, hydrogen atoms on −OH groups have migrated to their most adjacent carbon atom, making that carbon atom switch from sp^2 to sp^3 hybridization and breaking the aromaticity of the ring. Figure 3 pictorially describes how the migration occurs upon

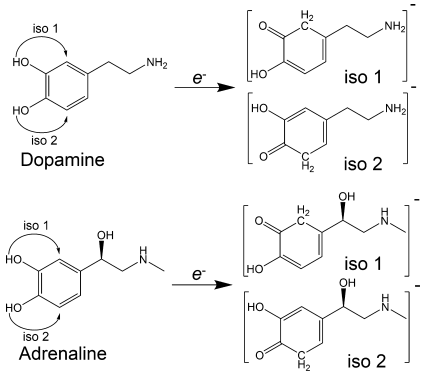

Figure 3. Schemes showing the formation of stable parent anions through hydrogen atom migration upon the attachment of an electron in dopamine and adrenaline.

attaching an electron. This structural change can be viewed as an enol−keto tautomerization from the neutral species to the anion, which tentatively explains why the H atom only migrates to the most adjacent carbon atom. We speculate that the tautomerization occurs because the resulting electron-withdrawing C═O group can better accommodate the excess electron. A similar process was observed in the acetoacetic acid parent anion.[26]

For dopamine, calculated VDE values (S_0) of anions iso 1 and iso 2 are 0.754 and 0.747 eV, both of which are in good agreement with the experimental value, 0.88 eV. The vertical transitions from the anion to the higher triplet states of the neutral species (T_1 and T_2) are tabulated in Table 1 and are also in good agreement with experiments. Since anion iso 2 is only 0.083 eV higher in energy than anion iso 1, we conclude that both anionic isomers probably coexist in the ion beam. The highest occupied molecular orbitals (HOMOs, Figure 2) where the excess electrons dwell show π^* antibonding orbitals delocalized on the sp^2 carbon atoms and the C═O groups for both iso 1 and iso 2, indicating that these anions are valence-bound. The sp^3 carbon atom that accepts the migrated H atom is excluded from the π conjugated system. For adrenaline, analogous observations can be made. The calculated VDE values as well as the calculated EBE values of the higher transitions for both of the anionic isomers are in good agreement with the experiment. Since anion iso 2 is only 0.024 eV higher in energy than iso 1, both isomers probably coexist in the ion beam.

Other structural possibilities of parent D^- and A^- anions include those in which both the H atoms migrate to the adjacent carbon atoms or in which each of the H atoms migrates to a non-adjacent carbon atom. The calculated VDE values of these isomers, however, do not coincide with any photoelectron features in the spectra.

DISCUSSION

Many early studies have revealed decreased dopamine levels in rat brains after various doses of radiation.[27−33] It was speculated that increased total numbers of dopamine receptors caused the decrease of dopamine concentrations.[27,32] However, the authors also stated that alternative hypotheses could not be excluded. Here, we raise the possibility that low-energy secondary electrons generated during radiation exposure[34] in the rats' brains could have produced the neurotransmitter parent anions being discussed here and that these and their further reactions might provide an alternative depletion channel for dopamine and adrenaline in vivo. Even though this work only focuses on dopamine and adrenaline, many other neurotransmitters, such as serotonin, tyrosine, DOPA, and phenylalanine, have similar structures where an acidic proton and a benzene ring are adjacent. Hence, we anticipate that similar parent anions could be formed in copious biomolecules upon the attachment of a low-energy electron. However, previous studies showed that under physoxia (physiological concentration of oxygen), secondary electrons are mostly converted into O_2^- and adrenaline reacts with hydroxyl radicals rather than with O_2^- as indicated by pulse radiolysis studies;[35] therefore, here, we do not claim that the parent anions in the current study play the role of a major depletion channel of the neurotransmitters in vivo.

Furthermore, secondary electrons have long been implicated in DNA damage either in the form of strand break or protonation following electron attachment.[36−39] The results of the present study are one more example that attention should be paid to the degradation of biomolecules due to the generation of secondary electrons by ionizing radiation.

CONCLUSIONS

Previously, neurotransmitters were known to only exist as closed-shell species, including neutral (zwitterionic) molecules and protonated or deprotonated ions. In this study, gas-phase anion photoelectron spectroscopy and DFT calculations have made possible the discovery of stable open-shell valence-bound neurotransmitter parent anions, that is, rare anionic tautomers, via an enol−keto type of tautomerization, which sheds light on a potential new depletion channel of these biomolecules caused by secondary electrons.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.9b06223.

Photoelectron spectra of the dopamine anion (D^-) and adrenaline anion (A^-), experimental and theoretical methods, and 3D coordinates of all the species calculated (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Siegel, G. J. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects;* Elsevier- Academic Press: London, 2006.

(2) Webster, R. A. *Neurotransmitters, Drugs and The Brain Function;* Wiley: New York, 2001.

(3) Grace, A. A. *Neuropsychopharmacology - The Fifth Generation of Progress;* Williams & Wilkins: New York, 2002.

(4) Chien, E. Y. T.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G. W.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; et al. Structure of the human dopamine D_3 receptor in complex with a D_2/D_3 selective antagonist. *Science* **2010**, *330*, 1091−1095.

(5) Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G. F.; Thian, F. S.; Kobilka, T. S.; Choi, H.-J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K.; et al. High-resolution crystal structure of an engineered human β_2-adrenergic G protein-coupled receptor. *Science* **2007**, *318*, 1258−1265.

(6) Warne, T.; Serrano-Vega, M. J.; Baker, J. G.; Moukhametzianov, R.; Edwards, P. C.; Henderson, R.; Leslie, A. G. W.; Tate, C. G.; Schertler, G. F. X. Structure of a β_1-adrenergic G-protein coupled receptor. *Nature* **2008**, *454*, 486−491.

(7) Rasmussen, S. G. F.; Choi, H.-J.; Rosenbaum, D. M.; Kobilka, T. S.; Thian, F. S.; Edwards, P. C.; Burghammer, M.; Ratnala, V. R. P.; Sanishvili, R.; Fischetti, R. F.; et al. Crystal structure of the human β_2-adrenergic G-protein-coupled receptor. *Nature* **2007**, *450*, 383−387.

(8) Carlsson, J.; Coleman, R. G.; Setola, V.; Irwin, J. J.; Fan, H.; Schlessinger, A.; Sali, A.; Roth, B. L.; Shoichet, B. K. Ligand discovery from a dopamine D_3 receptor homology model and crystal structure. *Nat. Chem. Biol.* **2011**, *7*, 769−778.

(9) Nagy, P. I.; Völgyi, G.; Takács-Novák, K. Tautomeric and conformational equilibria of tyramine and dopamine in aqueous solution. *Mol. Phys.* **2005**, *103*, 1589−1601.

(10) Macleod, N. A.; Simons, J. P. Infrared photodissociation spectroscopy of protonated neurotransmitters in the gas phase. *Mol. Phys.* **2007**, *105*, 689−700.

(11) Urban, J. J.; Cramer, C. J.; Famini, G. R. A computational study of solvent effects on the conformation of dopamine. *J. Am. Chem. Soc.* **1992**, *114*, 8226−8231.

(12) Hao, C.; March, R. E.; Croley, T. R.; Chen, S.; Legault, M. G.; Yang, P. Study of the neurotransmitter dopamine and the neurotoxin 6-hydroxydopamine by electrospray ionization coupled with tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 591−599.

(13) Jameson, R. F.; Hunter, G.; Kiss, T. A ^{1}H nuclear magnetic resonance study of the deprotonation of L-dopa and adrenaline. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1105−1110.

(14) Snoek, L. C.; Van Mourik, T.; Simons, J. P. Neurotransmitters in the gas phase: a computational and spectroscopic study of noradrenaline. *Mol. Phys.* **2003**, *101*, 1239−1248.

(15) ÇarÇabal, P.; Snoek, L. C.; Van Mourik, T. A computational and spectroscopic study of the gas-phase conformers of adrenaline. *Mol. Phys.* **2005**, *103*, 1633−1639.

(16) López, J. C.; Cortijo, V.; Blanco, S.; Alonso, J. L. Conformational study of 2-phenylethylamine by molecular-beam Fourier transform microwave spectroscopy. *Phys. Chem. Chem. Phys.* **2007**, *9*, 4521−4527.

(17) LeGreve, T. A.; Baquero, E. E.; Zwier, T. S. Infrared and ultraviolet spectral signatures and conformational preferences of jet-cooled serotonin. *J. Am. Chem. Soc.* **2007**, *129*, 4028−4038.

(18) Alonso, J. L.; Sanz, M. E.; López, J. C.; Cortijo, V. Conformational behavior of norephedrine, ephedrine, and pseudoephedrine. *J. Am. Chem. Soc.* **2009**, *131*, 4320−4326.

(19) Mitsuda, H.; Miyazaki, M.; Nielsen, I. B.; ÇarÇabal, P.; Dedonder, C.; Jouvet, C.; Ishiuchi, S.; Fujii, M. Evidence for catechol ring- induced conformational restriction in neurotransmitters. *J. Phys. Chem. Lett.* **2010**, *1*, 1130−1133.

(20) Ishiuchi, S.; Asakawa, T.; Mitsuda, H.; Miyazaki, M.; Chakraborty, S.; Fujii, M. Gas-phase spectroscopy of synephrine by laser desorption supersonic jet technique. *J. Phys Chem. A.* **2011**, *115*, 10363−10369.

(21) Cabezas, C.; Varela, M.; Peña, I.; López, J. C.; Alonso, J. L. The microwave spectrum of neurotransmitter serotonin. *Phys. Chem. Chem. Phys.* **2012**, *14*, 13618−13623.

(22) Cabezas, C.; Peña, I.; López, J. C.; Alonso, J. L. Seven conformers of neutral dopamine revealed in the gas phase. *J. Phys. Chem. Lett.* **2013**, *4*, 486−490.

(23) Nagy, P. I.; Alagona, G.; Ghio, C. Theoretical studies on the conformation of protonated dopamine in the gas phase and in aqueous solution. *J. Am. Chem. Soc.* **1999**, *121*, 4804−4815.

(24) Vorsa, V.; Willey, K. F.; Winograd, N. Photoionization of gas-phase versus ion-beam-desorbed dopamine with femtosecond laser pulses. *Anal. Chem.* **1999**, *71*, 574−581.

(25) van Mourik, T. The shape of neurotransmitters in the gas phase: A theoretical study of adrenaline, pseudoadrenaline, and hydrated adrenaline. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2827−2837.

(26) Keolopile, Z. G.; Gutowski, M.; Buonaugurio, A. M.; Collins, E.; Zhang, X.; Erb, J.; Lectka, T.; Bowen, K. H.; Allan, M. Importance of time scale and local environment in electron-driven proton transfer. The anion of acetoacetic acid. *J. Am. Chem. Soc.* **2015**, *137*, 14329−14340.

(27) Foulon, O.; Lalouette, F.; Lambert, F.; Martin, S.; Fatôme, M.; Martin, C. Effect of neutron-gamma radiation on dopamine and serotonin metabolism in the rat brain: A regional analysis. *J. Neurosci. Res.* **1999**, *55*, 770−775.

(28) Martin, C.; Rubio, I.; Fatome, M. Early and transient effects of neutron irradiation on dopamine receptors in the adult rat brain. *Neurosci. Lett.* **1993**, *155*, 77−80.

(29) Martin, C.; Rubio, I.; Burckhart, M. F.; Fatome, M. Effects of neutron-gamma irradiation on striatal D_1 and D_2 receptor distribution. *Neurosci. Lett.* **1994**, *178*, 107−110.

(30) Stepanović, S. R.; Nikolić, J. V. The effect of X-irradiation on the amount of dopamine in corpus striatum of the rat. *Experientia* **1979**, *35*, 111.

(31) Hunt, W. A.; Dalton, T. K.; Darden, J. H. Transient alterations in neurotransmitter activity in the caudate nucleus of rat brain after a high dose of ionizing radiation. *Radiat. Res.* **1979**, *80*, 556−562.

(32) Joyce, J. N.; Loeschen, S. K.; Marshall, J. F. Dopamine D-2 receptors in rat caudate-putamen: the lateral to medial gradient does not correspond to dopaminergic innervation. *Brain Res.* **1985**, *338*, 209−218.

(33) Kehr, W.; Carlsson, A.; Lindqvist, M. Catecholamine synthesis in rat brain after axotomy: Interaction between apomorphine and haloperidol. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1977**, *297*, 111−117.

(34) Beckham, W. A. Secondary electron scatter in radiotherapy: implications for treatment fields in proximity to the lens of the eye and the testes. *Phys. Med. Biol.* **1993**, *38*, 1013−1018.

(35) Bors, W.; Saran, M.; Michel, C.; Lengfelder, E.; Fuchs, C.; Spöttl, R. Pulse-radiolytic investigations of catechols and catecholamines. *Int. J. Radiat. Biol. Relat. Stud. Phys., Chem. Med.* **1975**, *28*, 353−371.

(36) Sanche, L. Low energy electron-driven damage in biomolecules. *Eur. Phys. J. D* **2005**, *35*, 367−390.

DOI: 10.1021/acs.jpcb.9b06223
J. Phys. Chem. B 2019, 123, 7695−7699

(37) Kumar, A.; Sevilla, M. D. Proton-coupled electron transfer in DNA on formation of radiation-produced ion radicals. *Chem. Rev.* **2010**, *110*, 7002−7023.
(38) Wang, W.; Marshall, M.; Collins, E.; Marquez, S.; Mu, C.; Bowen, K. H.; Zhang, X. Intramolecular electron-induced proton transfer and its correlation with excited-state intramolecular proton transfer. *Nat. Commun.* **2019**, *10*, 1170.
(39) Kohanoff, J.; McAllister, M.; Tribello, G. A.; Gu, B. Interactions between low energy electrons and DNA: a perspective from first-principles simulations. *J. Phys.: Condens. Matter* **2017**, *29*, 383001.

DOI: 10.1021/acs.jpcb.9b06223
J. Phys. Chem. B 2019, 123, 7695−7699